VISUAL INDICATORS OF INFECTION

BACKGROUND OF THE INVENTION

The invention concerns processes and products for the indication of infection such as in vaginitis and in wound care, particularly for those with diabetes, burns or immunodeficiencies.

Vaginal infections are the most common women's health problem, and commonly or medically referred to as vaginitis. Vaginal infections are the most frequent reason American women see their doctors, and account for more than 10 million office visits per year. Vaginitis is an inflammation of the vagina, and it is primarily in the forms of bacterial vaginosis, candida or yeast vaginitis and trichomonas vaginitis. The most prevalent type of infection is bacterial vaginosis, affecting up to 40 to 50% of American women of childbearing age.

Evidence suggests that pregnant women diagnosed with bacterial vaginosis have an elevated risk for preterm labor or premature rupture of the amniotic membrane, putting both mother and baby at higher risk. Additional possible adverse outcomes include an increased frequency of abnormal papanicolaou (Pap) smears, pelvic inflammatory disease and endometritis.

The most characteristic symptoms of vaginal infection are discharge, odor, irritation, and elevation of pH and amine(s) level. Various mono- and di-amines are produced by anaerobic bacteria, and result in odor in the vaginal fluid with bacterial vaginosis. Several detection methods are known for amines, these methods, however,

have complex multiple steps, require expensive instrumentation, or are time-consuming.

Diabetics, because of the nature of the disease, often have long convalescence periods from wounds. These wounds sometimes become infected, which eventually can be detected by their characteristic odor. Likewise, burn victims and those suffering from suppressed immune systems often have difficulty in healing and require extended periods for proper and complete wound healing, creating the possibility of infection.

Wounds most commonly associated with odor production include leg ulcers and fungating (cancerous) lesions of all types. The smell from these wounds is caused by amines and diamines that are produced by the metabolic processes of proteolytic bacteria together with short chain organic acids. As is the case with vaginal infections, rapid diagnosis and treatment of infected wounds is important for the immediate health of the person having the wound as well as for their long term recovery.

Bacteria and mold can cause infection in hosts other than the human body. In the field of food storage, for example, infection from mold and bacteria cause great economic loss each year.

It is clear that there exists a need for a process and product which allows for the rapid, inexpensive, and preferably non-invasive detection of infections.

SUMMARY OF THE INVENTION

In response to the foregoing difficulties encountered by those of skill in the art, we have developed a visual indicator test for the presence of amines generated by microbes, thus signaling the possible presence of infection. The indicator may be used in personal care products like, for example, feminine hygiene pads, and in health and hygiene products, for example, bandages. Disposable tests having test sites where sample materials may be contacted with the amine sensitive dye are also encompassed by this invention, as are non-

disposable tests suitable for use by medical professionals. The indicator of this invention may also be used to detect infection in grains, meats, legumes and other agricultural products and may also be used in veterinary applications to detect infections in animals.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a broken away drawing of a feminine hygiene product.

Figure 2 is a drawing of an adult incontinence product.

Figure 3 is a drawing of an absorbent underpant.

Figure 4 is a drawing of a bandage.

Figure 5 is a drawing of a standard curve for detection of TMA by ANB method.

Figure 6 is a drawing of a standard curve for detection of Putrescine by ANB method.

Figure 7 is a drawing of a standard curve for detection of Cadaverine by ANB method.

Figure 8 is a drawing of a disposable test device.

Figure 9 is a drawing of a bag for the storage of food.

DETAILED DESCRIPTION OF THE INVENTION

The present invention involves the visual indication of infection in vaginitis, in wounds and in other sites. Such an indicator may be placed in personal care products, e.g.; absorbent underpants, adult incontinence products, and feminine hygiene products. The indicator may also be incorporated into health and hygiene products like wound dressings or bandages. This indicator may also be included in a test device available over-the-counter or for tests to be administered by a health professional. It should be noted that although much

of the discussion that follows is directed to detection of infections of the human body, the indicators of this infection may be used to detect infections in anything subject to or capable of being infected by amine generating microbes. The indicator of the invention may thus be used in veterinary applications to detect infections in animals.

Bacterial vaginosis (BV) is the most common vaginal infection. Bacterial vaginosis (BV) affects up to 40 to 50% of American women of childbearing age, and is caused by increased anaerobic bacteria in the vagina that leads to characteristic amine production and odor. Various amines are produced by anaerobic bacteria, and these amines are released into the vaginal fluid in bacterial vaginosis (BV).

In addition to BV, trichomonas is a common sexually transmitted disease (STD) that affects 2 to 3 million Americans yearly. It is caused by a single-celled protozoan parasite called *trichomonas vaginalis*. Trichomoniasis is primarily an infection of the urogenital tract; the urethra is the most common site of infection in men, and the vagina is the most common site of infection in women. Though trichomoniasis occurs in men, they have no symptoms, whereas in women symptoms usually appear within four to 20 days of exposure. One of the diagnostic criteria for trichomonas is the detection of a fishy amine odor.

Vaginal yeast infection or vulvovaginal candidiasis is the second most common cause of vaginitis in the US and the most common cause in Europe. Doctors estimate that approximately 75 percent of all women will experience at least one symptomatic yeast infection during their lifetimes, and approximately 5 percent of women will have recurrent episodes. Candida albicans is the infecting agent in 80 to 90 percent of the yeast infected patients, and the doctor usually diagnoses yeast infection through microscopic examination of vaginal secretions for evidence of yeast forms such as candida albicans. Both pH and amine odors are not significantly changed in yeast vaginitis compared to normal women.

Both bacterial vaginosis and trichomoniasis produce tell-tale amine odors. In bacterial vaginosis, several members of anaerobic bacteria, prevotella, bacteroides, mobiluncus, and peptococcus, are present in large numbers in the vagina. Some of these organisms produce metabolic products such as amines, including trimethyl amine, putrescine, and cadaverine, which are responsible for the odor noticed by the affected patients. A "Whiff test" is routinely conducted for amine odors where enhanced odor is generated by adding strong alkali to the sample, however, this test must be professionally performed and the use of strong alkali is not applicable to personal care products due to the caustic nature of the chemical.

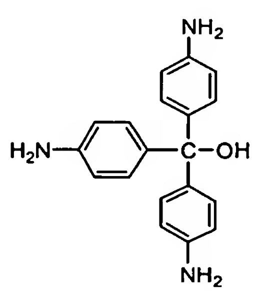
Other diagnosis factors such as pH and microscopic observation of clue cells (Coccobacilli) are indicative for bacterial vaginosis. Clue cells are observed in 90% of patients with bacterial vaginosis. Though elevated pH (at around 4.5 or greater) supports a diagnosis of bacterial vaginosis or trichomoniasis, certain other factors such as the use of antimicrobials and cervical discharge could be a misleading cause of the elevated pH. The normal vaginal environment is characterized by a dynamic interrelationship between *Lactobacillus acidophilus* and other endogenous flora, estrogen, glycogen, vaginal pH and the metabolic by-products of flora and pathogens. *Lactobacillus acidophilus* produces hydrogen peroxide, which is toxic to pathogens and keeps the healthy vaginal pH between 3.8 and 4.2.

Wounds most commonly associated with odor production include leg ulcers and fungating (cancerous) lesions of all types. The smell from these wounds is caused by volatile agents that include a mixture of amines and diamines such as cadaverine and putrescine that are produced by the metabolic processes of proteolytic bacteria together with short chain organic acids, (n-butyric, n-valeric, n-caproic, n-haptanoic and n-caprylic) produced by anaerobic bacteria. Organisms frequently isolated from malodorous wounds

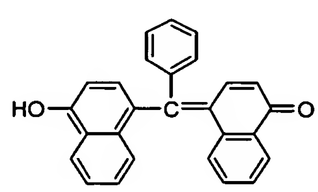
include anaerobes such as *Bacteroides* and *Clostridium* species, and numerous aerobic bacteria including *Proteus, Klebsiella* and *Pseudomonas.*

References report that the major odors detected from molds are identified as aliphatic alcohols (e.g. geosmine) and amines. An example of a mold is *Bacillus subtillis.* References further report identifying pyridine as well as simple amines in the odor given off by grains having bacteria contamination. An example of the bacteria is *Pseudomonas trifolii.* The economic loss cause by mold and bacteria each year is very large due to the destruction or spoilage of grain. Such microbes are also responsible for negative health effects when inhaled into the lungs.

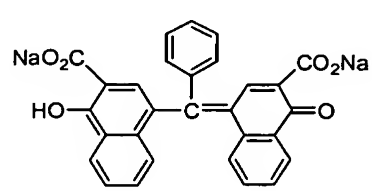
The inventors have discovered that an amine sensitive dye can be used to indicate the presence of amines in vaginal odor, in the odor from wounds, and in gases given off from other materials due to the presence of deleterious microbes, and thus to signal a possible infection. After an extensive search for amine-sensitive dyes, three dyes with different color spectrum were identified for the indication of amine odors, though others with similar characteristics regarding their reaction to amines may also be used. The three dyes identified were pararosaniline base (PAB), which turns from red to colorless, alpha-naphthol-benzein (ANB), which turns from orange/yellow to grey/black, and naphthochrome green (NCG) which turns pale yellow color to bluish green on exposure to amines. Their structures are shown below.



Pararosaniline Base (PAB)

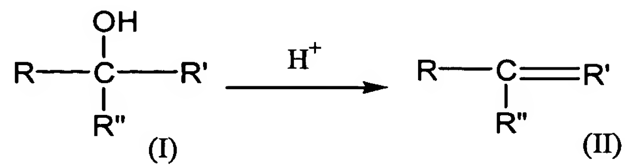


Alpha-Naphtholbenzein (ANB)

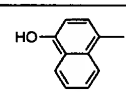
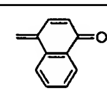
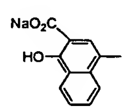
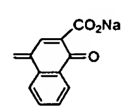


Naphthochrome Green (NCG)

Other indicating agents which are also suitable for the present invention can be represented by the following general formula (I) or (II):



where, R, R' and R" may each independently be a substituted aryl group, a napthyl, group, heteroaryl groups and hydrogen. More particularly the R, R' and R" groups may be:

Indicating Agent	**R**	**R'**	**R"**	**Indicating Agent for**
Michler's Hydrol (MH)	H	$(CH_3)_2NC_6H_5$-	$(CH_3)_2NC_6H_5$-	Ammonia, Amines, Diamines and Polyamines
Pararosaniline Base (PAB)	$(NH_2)C_6H_5$-	$(NH_2)C_6H_5$-	$(NH_2)C_6H_5$-	Ammonia, Amines, Diamines and Polyamines
Alpha-naphtholbenzein (ANB)	C_6H_5-			Ammonia, Amines, Diamines and Polyamines
Naphthochrome Green (NCG)	C_6H_5-			Ammonia, Amines, Diamines and Polyamines

Three common amines present when infection occurs are trimethylamine (TMA), putrescine (1, 4-diaminobutane or 1, 4 DAB) and cadaverine (1, 5-diamino pentane or 1, 5 DAP) and all are available in laboratory quantities from the Sigma-Aldrich Chemical Company of Milwaukee, WI, USA. A simple and rapid detection of cadaverine (1,5-DAP), Putrescine (1,4-DAB), and trimethylamine (TMA) was demonstrated by applying drops of these amines on alpha-naphtholbenzein (ANB) coated paper. These amines rapidly changed the dye color from orange yellow to grey/black. It was also found that these

amines could change the color of the dye when the amines were in the gaseous state when the dye did not come into contact with the liquid amine.

The amount of dye necessary to be effective, i.e., to change color and to be visible by the unaided eye, was found to be surprisingly small. An amount of dye between about 0.0001 and 20 weight percent on a dry basis was found to be sufficient. A more desirable amount of dye is between 0.001 and 10 weight percent on a dry basis and still more desirably between 0.01 and 1 weight percent on a dry basis.

The dye based indicator of this invention may be included in a myriad of products and devices for use by health professionals and lay people. It may be included in non-disposable and disposable test kits available over-the-counter or in a doctor's office. It may also be included in personal care products and in health and hygiene products.

Personal care products in which the inventive indicator may be placed include but are not limited to feminine hygiene products, incontinence products and absorbent underpants. The indicator may be placed, for example, in every product in a package containing multiple products or may be placed in every second, third or fifth product in the package, etcetera.

Feminine hygiene products include, for example, the pad shown in Figure 1. This pad 10 has a liquid impervious baffle 12 on the side away from the wearer. The baffle 12 is often made from a film like a polyethylene or polypropylene film. The layer closest to the wearer is the liner 14 and is a liquid pervious layer that is preferably soft and absorbent. Between the baffle 12 and liner 14 there may be a number of layers for different purposes, such as an absorbent core 16 designed to hold the majority of any liquid discharge. Other optional layers include transfer delay layers (not shown), and tissue wraps (not shown).

Incontinence products 30 as shown in Figure 2 likewise have a baffle or outer cover 32, an innermost liner 34 and various layers in between, like the absorbent core 36. More information concerning incontinence products may be found, for example, in US patents 4,940,464 and 4,938,753 which are incorporated herein in their entirety by reference

thereto for all purposes.

Absorbent underpants 50 as shown in Figure 3 have a baffle 52, liner 54 and absorbent core (not shown). A further discussion regarding absorbent underpants may be found, for example, in US patents 6,240,569 and 6,367,089 which are incorporated herein in their entirety by reference thereto for all purposes.

Since the inventors have found that the inventive indicator need not come into contact with the liquid amine to affect a color change, there is great flexibility in locating the indicator within a product. The indicator may be located anywhere it may be seen by a user during use or upon removal from service and prior to disposal. As shown in Figure 1 in the case of a feminine hygiene pad, the indicator 18 may be placed below the liner 14 on one end of the pad. In this location the indicator is not usually contacted directly by any discharge yet is still appropriately located so that it may be seen by the user upon removal. It should be noted that the indicator may be located in the "target area" (the area where discharges generally impact upon the product) of the pad if desired, or in other or multiple locations. The important factor is that it be visible to a user after amine exposure. The size of the indicator is likewise flexible, with the sole requirement being that it be of sufficient size to be seen by the unaided eye. Indicators as small as a few millimeters in area to as large as necessary may be produced, with the most likely sizes for personal care products being between 0.25 cm^2 and 5 cm^2, more desirably between about 0.5 cm^2 and 3 cm^2, still more desirably between 1 cm^2 and 2 cm^2.

Health and hygiene products in which the inventive indicator may be included include swabs, wet wipes, dry wipes, bandages, wound dressings, wraps and patches. As shown in Figure 4, bandages 70 and wound dressings commonly have a backing 78, an absorbent center portion 72 and an adhesive periphery 74 that is in contact with a wearer's skin. The backing 78 is the farthest layer from the wearer's skin and the absorbent portion 72 and the adhesive periphery 74 are placed on the body facing side of the backing 78. The indicator

of the invention may be placed within a bandage or wound dressing preferably so that it is visible from the side away from the body. In this manner the presence of infection may be indicated and observed without removal of the bandage from the wound. The indicator 80 may be placed under the backing 78 so that it is exposed to gaseous amine from the wound and so that it is visible through the backing. In this configuration it is necessary that the backing be sufficiently transparent or translucent so that the indicator is visible to the unaided eye. As in the case of personal care products, the size of the indicator must be sufficient so that it may be seen by the unaided eye. The same sizes used in personal care products are appropriate for health and hygiene products. Further discussion of bandages may be found in US Patent 6,576,575 which is incorporated herein in its entirety by reference thereto for all purposes.

The indicator may alternatively be placed within the health and hygiene product, for example, near the absorbent center portion 72 of a bandage, in a position wherein it will not be visible to the unaided eye. This location necessitates the removal of the bandage in order to determine if infection is present. In yet another alternative, the indicator may be a separate strip of material that may be inserted in the product in a position of the user's choosing. In the case of a wrap for a limb, for example, such a strip may be placed below the final few wraps of material so that the wrap may be unwound one or two turns to expose the indicator but need not be fully removed from the limb, i.e., the product must be partially removed to inspect the indicator. The dye indicator may also be incorporated into a wet or dry wipe for personal use so that the wipe or a portion of the wipe changes color upon use in the case of an infection.

The indicator of the invention may also be incorporated into a test kit or device. One example of such a device includes the indicator dye on, for example, a card. A sample of fluid or other material from the area to be tested may be obtained with an applicator such as a common cotton swab and the swab touched to the card in a test site where the dye has

been applied. A color change would indicate the presence of amines and thus possible infection. Such a test could be disposable after a number of tests as the example in Figure 8 illustrates. In Figure 8 the card 90 contains a number of circles or test sites 92 within which is printed the dye indicator of the invention. Upon contact with material from the swab 94 the dye will change color if amine is present. The presence of a plurality of test sites will allow for multiple individual tests, after which the card may be disposed of. If the test kit is provided with a plurality of test sites, they need not all contain the same dye but may also be provided with a plurality of dyes. Such a test would be useful not only to detect infection in the body but also in agricultural applications for the testing of meats, grains, legumes and other foods subject to infection and spoilage by microbes.

A non-disposible test suitable for use by medical professionals in an office setting is also included within the metes and bounds of the invention. For example, a known amount and concentration of the dye indicator may be placed in a microtiter plate well. A sample of the bodily fluid to be tested may be added to the dye indicator in the well, incubated for a short time and read at 650 nm using a microplate reader. The result may be compared to a known standard curve for the dye as shown for example in Figures 5, 6 and 7 for various dyes to determine the presence of absence of amine.

One example of a non-disposible test would use 190μl of 0.01, 0.05, or 0.1mg/ml ANB solution in acetonitrile in a microtiter plate well. A sample may be added to the plate and incubated at room temperature for less than a minute. The resultant color may be read at 650nm using a microplate reader and compared with the curve of Figure 5, for example, to determine the approximate amount of amine present. In regard to color testing, the most widely used color test is called CIELAB and is discussed in <u>Pocket Guide to Digital Printing</u> by F. Cost, Delmar Publishers, Albany, NY. ISBN 0-8273-7592-1 at pages 144 and 145. This method defines three variables, L*, a*, and b*, that correspond to three characteristics of perceived color based on the opponent theory of color

perception. The three variables have the following meaning:

L* = Lightness, ranging from 0 to 100. Where 0 = dark and 100= light,

a* = Red/green axis, ranging approximately from -100 to 100. Positive values are reddish and negative values are greenish.

b* = Yellow/blue axis, Ranging approximately from -100 to 100. Positive values are yellowish and negative values are blueish.

Because CIELAB color space is somewhat uniform, a single number can be calculated that represents the difference between two colors as perceived by a human being. This difference is termed ΔE and is calculated by taking the square root of the sum of the squares of the three differences (ΔL*, Δa*, and Δb*) between the two colors. In CIELAB color space, each ΔE unit is roughly a just-noticeable difference between two colors. So that the two colors have a difference of, for example, 4.4, the human eye can perceive the difference in color between the two colors. CIELAB is therefore a good measure for an objective device-independent color specification system that can be used as a reference color space for the purpose of color management and expression of changes in color.

Color intensities (L*a*b* values) herein were measured using a handheld spectrophotometer from Minolta Co. Ltd. of Osaka, Japan (Model # CM2600d). This instrument utilizes the D/8 geometry conforming to CIE No.15, ISO 7724/1, ASTME1164 and JIS Z8722-1982 (diffused illumination/ 8 degree viewing system. The D65 light reflected by the specimen surface at an angle of 8 degrees to the normal of the surface is received by the specimen-measuring optical system. Absorbance readings herein were measured using a microplate reader from Dynex Technologies of Chantilly, Virginia (Model # MRX).

Figure 9 is a drawing of a bag useful for the storage of food. Such bags 100 are generally transparent so that the contents may be viewed without opening the bag and have

a means for closure on one side 102. The indicator 104 of the invention may be placed on the inner surface 106 of such a transparent bag so that it is visible to a user, thus alerting the user to spoilage of the contents of the bag. If the bag is not transparent, the bag may be opened to inspect the dye indicator 104. The indicator must be of an area sufficient to be visible to the unaided eye.

The indicator dyes are generally dissolved in a solvent before application. Suitable solvents include acetonitrile, dimethylsulfoxide (DMSO), ethyl alcohol, dimethylformamide (DMF) and other polar organic solvents. The amount of dye to be dissolved is desirably between about 0.01 and 0.1 mg/ml solvent. The dissolved dye may be coated onto a fabric layer, dried, and the dried layer placed in the product as the "patch" or "insert" discussed above. Alternatively, the indicating solution may be coated onto an existing layer within the product, like the absorbent core or liner in the case of a personal care product, for example, and allowed to dry. The indicator may be coated onto the substrate layer by the dip and squeeze method, which entails dipping the layer into a solution having the absorbent and binder, squeezing out the excess, and drying. The indicator may be applied to the substrate by placing a few drops of the dye solution on the desired area and allowing the substrate to air-dry, as for example, in the case of the disposable card of Figure 8. It is sufficient that the dye be placed in a location such that its color change is visible the unaided eye.

Substrates suitable for coating with the indicators of the invention include cellulose, woven and nonwoven fabric, cotton, silk, rayon, glass fiber, films like polypropylene/polyethylene film, tissues, paper towels, coform materials, airlaid materials, wet-laid materials, bonded-carded webs and so forth. Nonexclusive examples of substrates may be found in US patents 4,775,582 and 4,853,281, 4,833,003, and 4,511,488, all assigned to the Kimberly-Clark Corporation.

A nonwoven fabric may be made according to processes like spunbonding, meltblowing, airlaying, bonding and carding, and so forth. Nonwoven fabrics may be

made from thermoplastic resins including, but not limited to polyesters, nylons, and polyolefins. Olefins include ethylene, propylene, butylenes, isoprene and so forth, as well as combinations thereof.

The term "coform" means a process in which at least one meltblown diehead is arranged near a chute through which other materials are added to the web while it is forming. Such other materials may be pulp, superabsorbent particles, natural polymers (for example, rayon or cotton fibers) and/or synthetic polymers (for example, polypropylene or polyester) fibers, for example, where the fibers may be of staple length. Coform processes are shown in commonly assigned US Patents 4,818,464 to Lau and 4,100,324 to Anderson et al. Webs produced by the coform process are generally referred to as coform materials.

A bonded carded web is made from staple fibers which are sent through a combing or carding unit, which breaks apart and aligns the staple fibers in the machine direction to form a generally machine direction-oriented fibrous nonwoven web. Once the web is formed, it then is bonded by one or more of several methods such as powder bonding, pattern bonding, through air bonding and ultrasonic bonding.

In the airlaying process, bundles of small fibers having typical lengths ranging from about 3 to about 52 millimeters (mm) are separated and entrained in an air supply and then deposited onto a forming screen, usually with the assistance of a vacuum supply. The randomly deposited fibers then are bonded to one another. Examples of airlaid teachings include the DanWeb process as described in US patent 4,640,810 to Laursen et al. and assigned to Scan Web of North America Inc, the Kroyer process as described in US patent 4,494,278 to Kroyer et al. and US patent 5,527,171 to Soerensen assigned to Niro Separation a/s, the method of US patent 4,375,448 to Appel et al assigned to Kimberly-Clark Corporation, or other similar methods.

Additional substrates upon which the amine sensitive dye may be coated include silica gels, latex particles and nanoparticles. The visual indicator dye can be incorporated

into particles of various size from millimeter to nanometer by passive adsorption or covalent coupling. The adsorption of dye onto particles can be monitored by measuring the color intensity changes for before and after coating to particles with spectrophotometer. In order to determine the effectiveness of the adsorption of the dye onto various particles, ANB dye at a concentration of 0.001-5000 milligrams was coated onto silica gel (particle size ~millimeter) and latex particles (particle size ~ microns). The results are indicated as follows.

Substrate	ANB Dye Color Intensity at 450nm Before Coating to Particle	ANB Dye Color Intensity at 450nm After Coating to Particle
Silica Gel (200-400 mesh, 60Å)	0.450 (solution diluted to 10 times)	0.418 (solution diluted to 10 times)
Latex Particles ((P(S/V-COOH), 0.3 micron size)	3.362	0.968

These results indicate that the dye was coated onto the particles.

The following examples aid in understanding the invention. Note that the comparative example is not an example of the invention.

Example 1

10μl of 10mg/ml stock solution of ANB in acetonitrile was spotted onto a SCOTT® paper towel and air dried. Each of the three amines (TMA, 1,4-DAB, and 1,5-DAP) was individually applied to the ANB-coated paper towel by dipping a cotton swab having the 10mg/ml stock concentration of the amine in acetonitrile. This resulted in a rapid color

change with each of the amines.

Example 2

A visual indicator was incorporated into a feminine hygiene pad as a small strip of tissue and demonstrated to be sensitive to the amine-based vaginal odors.

ANB dye at a concentration of 50 – 5000 micrograms was coated onto cellulose towel substrate and allowed to air dry, producing an orange color. Small strips (1cm x 2cm) of the towel were then inserted into the pad at one end under the body side liner. The orange colored strip was still quite visible. The pad was then insulted with 10 microliters of a 10mg/ml TMA amine solution at a concentration of 100 micrograms placed in the target area of the pad, and then the pad was placed into a 1 quart, glass jar and sealed. The liquid amine did not directly touch the dye. The orange color of the cellulose strip rapidly changed color to a grey/black color, indicating the presence of amines and therefore serving as an alert signal for potential vaginitis.

This experiment demonstrates the potential application for the visual indicator to detect the amine odor from the center of the pad and act as visual alert to the user. It is noted that the strip did not come in contact with the odorous liquid, and the observed color change was therefore due to the gaseous odor.

In order to test the indicator of the invention, dye in solution was placed on substrates, exposed to various amines and the color tested using the testing devices described above, in the following Examples.

Example 3

A standard curve was generated using various trimethylamine (TMA) concentrations by ANB method (see Figure 5). In Figure 5, the x-axis is the concentration of TMA in micrograms from 0 to 175 and the y-axis is the absorbance at 650 nm. The method involves the following steps: 10μl of each concentration of TMA stock solution of 0, 0.25,

0.5, 1, 2, 4, 8, 16mg/ml (corresponding to 0, 250, 500, 1000, 2000, 4000, 8000, and 16000 ppm) in water was placed in a microtiter plate well, and mixed with 190μl of 0.01, 0.05, or 0.1mg/ml ANB solution in acetonitrile. The wells were incubated at room temperature for less than a minute, and read at the plate at 650nm using microplate reader. The upper line in the graph is the 0.1 mg/ml ANB solution, the middle line is the 0.05 mg/ml ANB solution and the lower line is the 0.01 mg/ml ANB solution.

The sensitivity of amine detection was very high according to this method, and it was shown that the sensitivity could be altered by varying the dye concentration.

Example 4

A standard curve was generated using various Putrescine (or 1,4-Diaminobutane, 1,4-DAB) concentrations by ANB method (see Figure 6). In Figure 6, the x-axis is the concentration of 1,4-DAB in milligrams from 0 to 0.6 and the y-axis is the absorbance at 650 nm. The method involves the following steps: 50μl of each concentration of 1,4-DAB stock solution of 0, 0.15, 0.3, 0.6, 1.25, 2.5, 5, and 10 mg/ml (corresponding to 0, 150, 300, 600, 1250, 2000, 5000, and 10000ppm) in acetonitrile was placed in microtiter plate well, and mixed with 150μl of 0.01, 0.05, or 0.1mg/ml ANB solution in acetonitrile. The wells were incubated at room temperature for less than a minute, and read at the plate at 650nm using microplate reader. The upper line in the graph is the 0.1 mg/ml ANB solution, the middle line is the 0.05 mg/ml ANB solution and the lower line is the 0.01 mg/ml ANB solution.

The sensitivity of amine detection was very high according to this method, and it was shown that the sensitivity could be altered by varying the dye concentration.

Example 5

A standard curve was generated using various concentration of Cadaverine (or 1,5-Diaminopentane, 1,5-DAP) concentrations by ANB method (see Figure 7). In Figure 7, the x-axis is the concentration of 1,5-DAP in milligrams from 0 to 0.6 and the y-axis is the

absorbance at 650 nm. The method involves the following steps: 50μl of each concentration of 1,5-DAP stock solution of 0, 0.15, 0.3, 0.6, 1.25, 2.5, 5, 10mg/ml (corresponding to 0, 150, 300, 600, 1250, 2000, 5000, and 10000ppm) in acetonitrile was placed in microtiter plate well, and mixed with 150μl of 0.01, 0.05, or 0.1mg/ml ANB solution in acetonitrile. The wells were incubated at room temperature for less than a minute, and read the plate at 650nm using microplate reader. The upper line in the graph is the 0.1 mg/ml ANB solution, the middle line is the 0.05 mg/ml ANB solution and the lower line is the 0.01 mg/ml ANB solution.

The sensitivity of amine detection was very high according to this method, and it was shown that the sensitivity could be altered by varying the dye concentration.

Comparative Example 1

A commercially available test, the QuickVue® Advance pH and amines test, from Quidel Corporation of San Diego, CA, USA was tested. This product is available in individually foil-wrapped tests with sterile amine-controlled cotton swabs for retrieving samples to be tested. The QuickVue® test involves the use of pH sensitive dyes combined with alkali to detect amines. The alkali serves to make amine more nucleophilic in order to react with the dye. The test contains a strong base (10 weight percent potassium hydroxide) to detect amines and so may not be suitable for use in personal care products. The QuickVue® test is designed for use by the medical professional in a clinical environment. The QuickVue® test yields a result in about 2 minutes and was found to be sensitive to an amine (1,4 DAB) concentration of 25 micrograms.

As will be appreciated by those skilled in the art, changes and variations to the invention are considered to be within the ability of those skilled in the art. Examples of such changes are contained in the patents identified above, each of which is incorporated herein by reference in its entirety to the extent it is consistent with this specification. Such

changes and variations are intended by the inventors to be within the scope of the invention. It is also to be understood that the scope of the present invention is not to be interpreted as limited to the specific embodiments disclosed herein, but only in accordance with the appended claims when read in light of the foregoing disclosure.